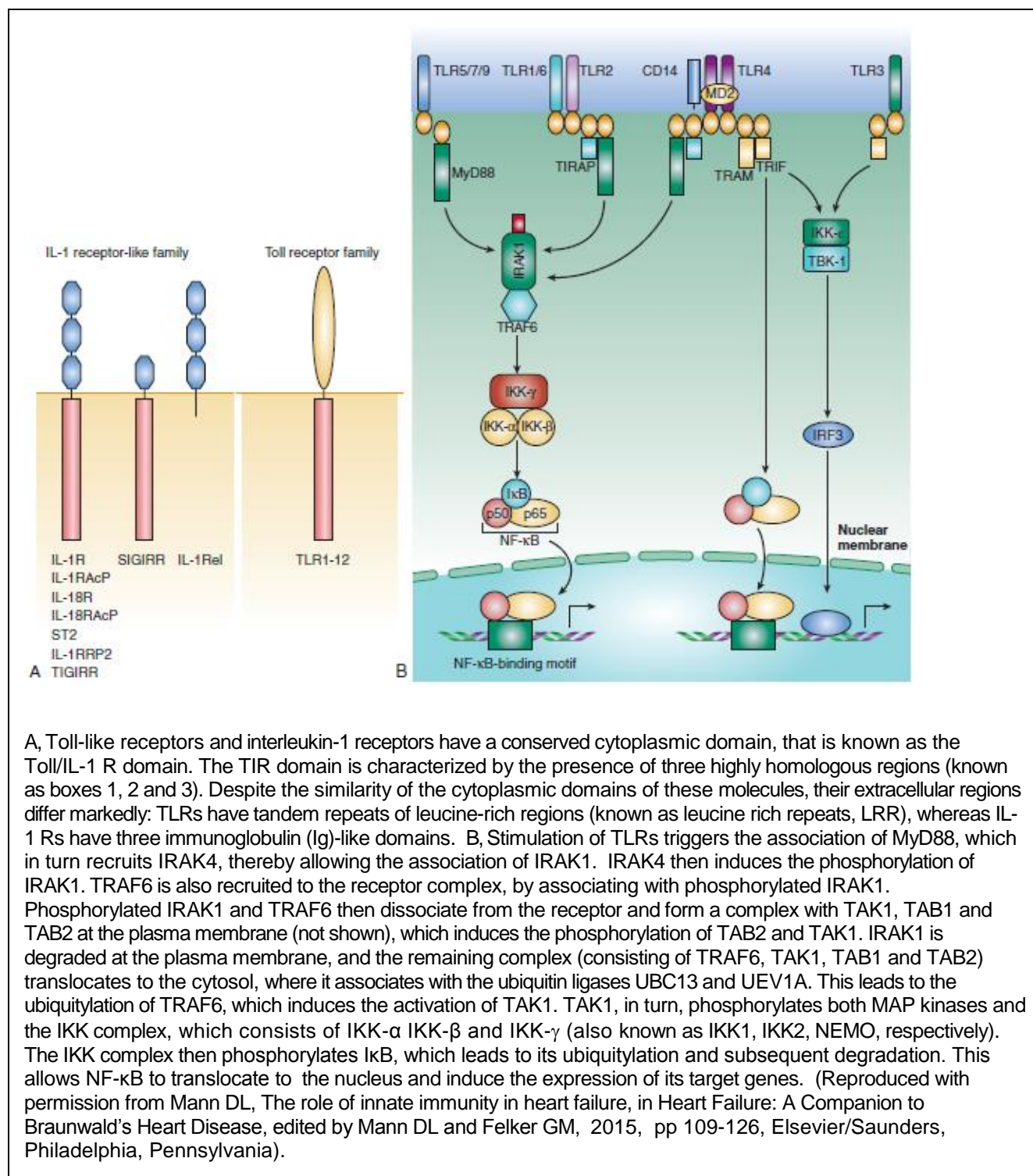


## SUPPLEMENTAL MATERIAL FOR INNATE IMMUNITY AND THE FAILING HEART: THE CYTOKINE HYPOTHESIS REVISITED

### TOLL-LIKE RECEPTOR SIGNALING PATHWAYS



As shown in panel A of the Figure, the signaling pathway that is used by the TLR family of receptors is highly homologous to that of the IL-1 receptor (IL-1R) family (see below). TLRs are type 1 membrane-spanning receptors that have a leucine-rich repeat extracellular motif and an intracellular signaling motif that is similar to interleukin (IL-1). With the exception of TLR3, all TLRs interact with an adaptor protein termed MyD88 (myeloid differentiation factor 88) via their Toll Interleukin Receptor (TIR) domains (panel B). MyD88-dependent signaling through TLR2 and TLR4 requires an adaptor protein termed TIRAP (TIR domain-containing adaptor protein) to initiate signaling. When stimulated, MyD88 sequentially recruits IL-1 receptor associated kinases 4, 1 and 2 (IRAK4, IRAK1 and IRAK2) to the receptor complex. Phosphorylation of IRAK1 on serine/threonine residues by IRAK4 results in recruitment of tumor necrosis receptor associated factor 6 (TRAF6) to the complex, which is responsible for early responses in response to TLR signaling (see reference <sup>1</sup> for more details on TLR signaling).

## **PROINFLAMMATORY CYTOKINES**

### **Tumor Necrosis Factor Superfamily**

The tumor necrosis factor (TNF) superfamily consists of 19 well-characterized ligands (TNFSF) and 34 TNF superfamily receptors (TNFRSF). Members of the TNF superfamily of ligands and receptors are expressed in a broad variety of cell types, including myocardial cells.<sup>2</sup> Notably, all members of the TNF superfamily exhibit pro-inflammatory activity. Of note, recent studies have identified a potential role for TNF superfamily ligands/receptors in terms of mediating inflammatory responses in the heart, including TNF/TNFR1, TNFR2 (TNFSF2/TNFRSF1A, TNFRSF1B), FasL/Fas (TNFSF6/TNFRSF6), TWEAK (tumor necrosis factor-like weak inducer of apoptosis) /TWEAKR (TNFSF12/TNFRSF12),<sup>3</sup> and RANKL (Receptor activator of NF- $\kappa$ B ligand)/ RANK (TNFSF11/TNFRSF11A).<sup>4</sup> In contrast to FasL, TNF, TWEAK and RANKL signal through TNFSF receptors that engage a common scaffolding protein

termed TNF receptor associated factor 2 (TRAF2), that is recruited to the TNFSF receptors following engagement of their cognate ligands. In contrast to TNF, TWEAK and RANKL, cardiac-restricted overexpression of FasL (TNFSF6) does not lead to dilated cardiomyopathy. Notably, TNF, TWEAK and RANKL signal through TNFSF receptors that engage a common scaffolding protein termed TNF receptor associated factor 2 (TRAF2), that is recruited to the TNFSF receptors following engagement of their cognate ligands. Cardiac restricted expression of TRAF2 provokes a dilated cardiac phenotype, that phenocopies cardiac restricted overexpression of TNF, and is mediated, at least in part, through NF- $\kappa$ B.<sup>5</sup>

### **Interleukin-1 Family**

Although the original IL-1 family (IL-1F) consisted of IL-1 $\alpha$  (IL-1 F1) and IL-1 $\beta$  (IL-1 F2), the IL-1 family has now expanded to include seven ligands with agonist activity (IL-1 $\alpha$  and IL-1 $\beta$ , IL-18 (IL-1F4), IL-33 (IL-1F11), IL-36 $\alpha$  (IL-1F6), IL-36 $\beta$  (IL-1F7), IL-36 $\gamma$  (IL-1F8), three receptor antagonists (IL-1Ra [IL-F3], IL-36Ra [IL-F5], IL-38 [IL-F10]), and an anti-inflammatory cytokine (IL-37 [IL-10]). Members of the IL-1 Receptor (IL-1R) family include six receptor chains forming four signaling receptor complexes, two decoy receptors (IL-1R2, IL-18BP), and two negative regulators (TIR8 or SIGIRR [IL-1R8], IL-1RAcPb).<sup>6</sup> Similar to TNF, IL-1 $\beta$  and IL-18 appears to be synthesized within the myocardium in response to stressful environmental stimuli, and both IL-1 $\beta$  mRNA and protein have been detected in the hearts of patients with dilated cardiomyopathy.<sup>7</sup> Moreover, in vivo studies have shown specific blockade of IL-18 using IL-18 binding protein improves contractile function in human atrial tissue following ischemia reperfusion injury,<sup>8</sup> as well as lipopolysaccharide-induced LV dysfunction in experimental animals.<sup>8</sup>

### **Interleukin-6 (IL-6) Family**

Based on their functional redundancy, structural similarity, and use of a common signaling receptor, interleukin-6 (IL-6), leukemia inhibitory factor (LIF), cardiotrophin-1 (CT-1),

ciliary neurotrophic factor (CNTF), interleukin-11 (IL-11), and oncostatin M (OSM) are considered to represent the "IL-6 family" of cytokines. Inclusion in the IL-6 family is based on a helical cytokine structure and receptor subunit makeup. The IL-6 family of cytokines triggers downstream signaling pathways in multiple cell types, including cardiac myocytes, either through the homodimerization of the gp130 receptor or through the heterodimerization of gp130 with a related transmembrane receptor. All IL-6 type cytokines potentially activate STAT3, and to a lesser extent STAT1 through their common gp130 subunits. The specificity of cytokine signaling within the IL-6 family is determined by the composition of the cytoplasmic domains associated with the signal-competent receptor complex.<sup>9</sup> The suppressor of cytokine signaling (SOCS) (also referred to as cytokine-inducible SH2 proteins [CIS]) are a family of specific negative regulatory feedback elements of JAK/STAT signaling. Expression of some SOCS family members is regulated transcriptionally by STATs, thereby acting as a negative feedback loop for JAK-STAT signaling. Both SOCS-1 and SOCS-3 interact with the kinase domain of various JAK proteins, thereby preventing STAT phosphorylation. Previous clinical studies showed that the plasma level of IL-6, CT-1, LIF and gp130 are elevated in patients with advanced heart failure and that high levels are associated with a poor prognosis for heart failure patients.<sup>10, 11</sup>

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